INSTRUCTIONS FOR USE

UREA 40% SOLUTION

Liquid supplement

1 - INTENDED USE

In vitro diagnostic. Urea solution, added to Urea Broth Base or Urea Agar Base, is used for the determination of urease enzyme as an aid for the differentiation of microorganisms.

2 - COMPOSITION - VIAL CONTENTS

	REF 42211601	REF 4240096
Urea	20 g	2 g
Purified water	50 mL	5 mL

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Urea 40% Solution is used as a supplement of Urea Broth Base or Urea Agar Base. The complete medium Urea Broth conforms to the formulation of Rustigian and Stuart.[1,2] The complete medium Urea Agar conforms to Christensen's[2] modification of the Rustigian and Stuart[1,2] formula and to the formulation recommended by ISO 6579[4] and FDA BAM[5]
Both media are intended for the determination of the urease enzyme (urea amidohydrolase), as an aid for the differentiation of members of the *Enterobacteriaceae* (Urea Broth) and *Enterobacteriaceae* and other microorganisms (Urea Agar)[6,7]
The urease test with Urea Agar is one of the tests recommended by ISO 6579[3] for the identification of *Salmonella* spp.
Urea, added to the base medium, is hydrolysed by the microorganisms with the formation of ammonium ions and subsequent alkaline reaction that induces the purple-red turn of phenol red when the pH of the medium exceeds 8.1.[6]

4- DIRECTIONS

Prepare 950 mL of Urea Broth Base (Stuart), REF 402180 or 950 mL of Urea Agar Base (Christensen), REF 402175, autoclaved and cooled to 47-50°C. Under aseptic conditions add 50 mL of Urea 40% Solution (REF 42211601 or 4240096).
Complete Urea Broth: mix well and dispense the medium in quantities of 3-5 mL into sterile tubes, under aseptic conditions.
Complete Urea Agar: mix well and dispense the medium in quantities of 10 mL into sterile tubes under aseptic conditions. Cool in slanted position (long slant/short butt).

5 - PHYSICAL CHARACTERISTICS

Solution appearance colourless, limpid

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Urea 40% Solution	Liquid supplement	42211601 4240096	50 mL 10 x 5 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Urea Broth Base (REF 402180), Urea Agar Base (REF 402175) autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and swabs, sterile tubes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Urea Broth and Urea Agar, supplemented with urea solution, shall not be used for the direct inoculation of clinical specimens. The samples consist of isolates from pure culture grown on solid medium.

9 - TEST PROCEDURE

Urea Broth
Inoculate the broth heavily with 3 loopfuls (2 mm loop) from an 18-24 h pure culture obtained on TSI or another appropriate medium. Shake the tube gently to suspend the colonies. Incubate the tubes with loosened caps at 35-37°C in an incubator or water bath for 8-48 hours. Examine broths for colour change at 2, 4, 6, 18, 24, and 48 hours of incubation.
Urea Agar
Inoculate the slope heavily (from an 18-24 hours pure culture) over the entire surface by streaking the surface of the agar. Do not stab the butt; it serves as a colour control.
Incubate inoculated tube with loosened cap at 35-37°C and observe the colour change of the medium to red-violet after 2, 4, 6, 18, 24 hours and daily for a total incubation time of 6 days.
Method recommended by ISO 6579[3]: streak the agar slant surface and incubate at 37 °C for up to 24 h. The positive reaction is often apparent after 2 h to 4 h.

10 - READING AND INTERPRETATION

After incubation, observe the colour change of the media.
The positive test (urea hydrolysis) is indicated by a bright pink (fuchsia) colour.
The negative test is indicated by the unchanged colour of the medium (e.g., *Salmonella* spp.).
Consult also the Information for Use of Urea Broth Base (REF 402180) and Urea Agar Base (REF 402175).

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control testing in accordance with the local applicable regulations, in

compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]
Urease positive control: *P.vulgaris* ATCC 9484
Urease negative control: *E.coli* ATCC 25922

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Urea 40% Solution added to dehydrated Urea Broth Base (REF 402180) and Urea Agar Base is tested performances characteristics comparing the results with a previously approved Reference Batch with the following strains. Urea Agar: *P.mirabilis* ATCC 10005, *P.stuardi* ATCC 33672, *P.rettgeri* ATCC 39944, *K,pneumoniae* ATCC 27736 and *E.coli* ATCC 25926; Urea Agar: *P.morgani* CB 118, *P,vulgaris* ATCC 9484, *K,pneumoniae* ATCC 27736, S,Typhimurium ATCC 14028 . Colour change of the medium is observed after 2, 6, 24, 48 hours of incubation at 35-37°C. Urease positive strains in Urea Broth: *P.mirabilis*, *P.stuardi*, *P.rettgeri*; urease positive strains in Urea Agar: *P.morgani, P,vulgaris*, *K,pneumoniae* ATCC 27736.

13 - LIMITATIONS OF THE METHOD

Urea Broth

- Urea Broth a highly buffered medium that requires large amounts of ammonia to raise the pH to 8.1 resulting in a colour change. Slowly and weakly urease-positive strains, due to the low concentration of yeast extract and a strong buffering system, appear as urease negative on Urea Broth (Stuart).[6,7]
- Purple-red turning occurs when the pH reaches 8.1; inoculation significantly affects the time required by the bacterial strain to develop these alkalinity values and thus a positive reaction.[6]
- The rate of urease reaction is also affected by the volume of liquid medium in the tube; Stuart et al.[1] report that with increasing volumes of 1.5 mL, 3 mL, 4.5 mL, 6 mL, for the same inoculum, the time of development of the positive reaction increases and that the minimum volume for the test is 1.5 ml.
- Urea Broth tubes are not suitable for quantitative evaluation of urea hydrolysis.

Urea Agar

- The urea test is based on the alkalinisation of the culture medium and is therefore not specific for the urease enzyme. The utilisation of peptones, especially on the slope, for example by *P.aeruginosa*, may raise the pH to alkalinity, resulting in false positive reactions. To eliminate possible false positive, run a control test using the same strain and the test medium without urea.[6]
- Urease positive *Proteus* spp. cause a rapid alkalinisation of the medium. For the results to be valid for the detection of *Proteae*, the results must be read within the first 2-6 hours interval of incubation. *C.freundii* and *K.pneumoniae* convert Urea Agar within 24-48 hours. This medium detects rapid urease activity only of urease positive *Proteae*.[6]
- Do not inoculate Urea Agar slopes with cultures obtained from liquid media.
- Prolonged incubations could give rise to false positive results due to urea autolysis; when a long incubation is expected, incubate also an un-inoculated tube to verify the occurrence of urea autolysis.
- Even if the microbial colonies are differentiated on the basis of urea hydrolysis, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- The culture medium and the supplement are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- The supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The supplement is not classified as dangerous according to current European legislation.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the production process of prepared media.
- The supplement is sterilized by membrane filtration.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened the solution should be used immediately. Before use, examine the solution and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Rustigian R, Stuart CA. Decomposition of urea by *Proteus*. Proc Soc Exp Biol Med. 1941; 47:108-112
2. Stuart CA, Van Stratum E, Rustigian R. Further Studies on Urease Production by Proteus and Related Organisms. J Bact 1945; 48:437
3. Christensen WB. Urea Decomposition as a Means of Differentiating *Proteus* and *Paracolon* Cultures from Each Other and from *Salmonella* and *Shigella* Types. J Bact 1946; 52:461-466
4. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: *Salmonella*. Rev 07/2020
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins;
7. Public Health England. UK Standards for Microbiology Investigations. Urease test. TP 36, Issue n° 4, 04/2019

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/03
Revision 2	Removal of obsolete classification, clarification on inoculation and reading methods	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.